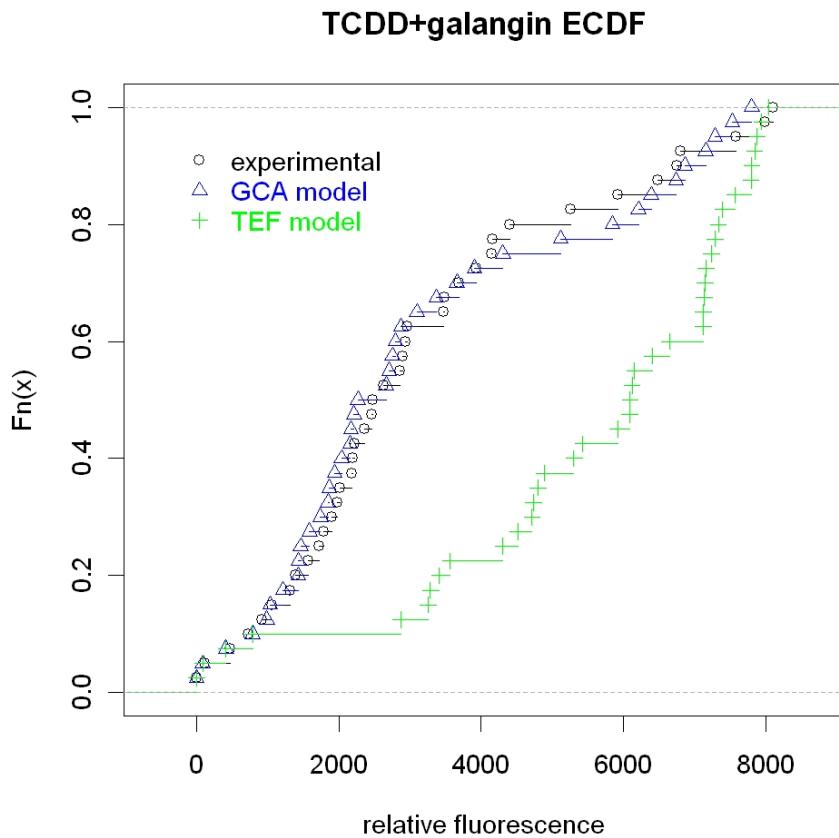


Supplemental Material

Measurement of AhR activation (H1G1 assay). H1G1.1c3 cells were maintained and prepared for experiments largely as described previously (Nagy et al. 2002). H1G1.1c3 cells were plated at a concentration of 2×10^4 cells per well in 200 μl of medium (α MEM, 10% FBS, 50 units/ml penicillin/streptomycin) containing G418 (968 mg/l). The cells were incubated at 37°C for 24 hrs. The medium was removed and replaced with 100 μl of non-selective medium prior to application of the test compounds. Test compounds stock solutions were prepared in DMSO and were diluted in DMSO. Each experiment used an array of 7 plates that was treated with combinations of compounds. Vehicle (DMSO, 0.5%), a partial agonist (PCB105 10^{-9} – 10^{-5} M or galangin 10^{-7} – 3×10^{-5} M), an antagonist (DIM, 5×10^{-6} – 3×10^{-5} M), or a full agonist (PCB126, 10^{-10} – 10^{-8} M) was applied at a single concentration over the full plate, excluding 2 columns that were left untreated. Either vehicle or an increasing concentration of the experimental compound was applied to each plate. This was followed immediately by dosing with either vehicle (DMSO, 0.5%) or a TCDD (10^{-15} – 5×10^{-10} M) or a TCDF (10^{-14} – 10^{-8} M) standard curve, replicated in each of eight rows per plate. The plates were incubated at 33°C for 24 hrs, after which EGFP fluorescence was read with a fluorometric plate reader (Synergy2, Biotek Instruments). The excitation and emission wavelengths were 485 nm (20 nm bandwidth) and 530 nm (25nm bandwidth). For each plate, the fluorescence measured in wells with untreated cells was subtracted from fluorescence in the experimental wells. Because gain settings on the plate reader varied, we report only relative fluorescence values. The eight replicates of each combination within a plate were averaged in each experiment. Each experiment was repeated at least 3 times.

General toxicity was assessed following the fluorescence measurement by adding 20 μl

of a 5 mg/ml solution of thiazolyl blue tertazolium bromide (MTT) in PBS per well, incubating for 4-6 hrs at 37°C and reading the absorbance at 570 nm. A significant reduction in the absorbance in comparison to vehicle-treated cells was used as an indicator of toxicity. Significance was tested by ANOVA, with Dunnett's test for multiple comparisons ($p < 0.05$). Only combinations that had no significant effect on MTT absorbance were used in the model analyses.



Supplemental Figure 1. Empirical cumulative distribution function of experimental data (circles), TEF model (plus symbols), and GCA model (triangles) for the TCDD+galangin combination. The ECDF plots the proportion of the response surface (y axis) that is at or below each value of the effect (x axis, measured in fluorescence units).

Supplemental Table 1. Results for mixtures of TCDF and PCB126. Mean and standard error (SE) of naïve-corrected fluorescence in the H1G1 assay (n=4).

| | | PCB126 [M] | | | | | | | | | | | |
|------|-------|------------|-----|-------|-----|-------|-----|-------|-----|-------|-----|-------|-----|
| | | Vh | | 1E-10 | | 5E-10 | | 1E-09 | | 5E-09 | | 1E-08 | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Vh | | 448 | 60 | 2302 | 200 | 4666 | 562 | 5940 | 430 | 7275 | 508 | 8382 | 144 |
| | 1E-13 | 597 | 71 | 2418 | 268 | 4736 | 381 | 6372 | 620 | 7322 | 464 | 8285 | 141 |
| | 1E-12 | 759 | 104 | 2672 | 211 | 4765 | 492 | 6340 | 459 | 7266 | 547 | 8326 | 208 |
| | 1E-11 | 2651 | 314 | 3816 | 352 | 5060 | 406 | 6570 | 492 | 7256 | 547 | 8351 | 427 |
| TCDF | 5E-11 | 5384 | 378 | 5221 | 396 | 5881 | 402 | 6801 | 423 | 7448 | 438 | 8645 | 305 |
| [M] | 1E-10 | 6393 | 492 | 5989 | 488 | 6622 | 642 | 7118 | 377 | 7350 | 312 | 8373 | 245 |
| | 5E-10 | 8012 | 279 | 7480 | 365 | 7769 | 681 | 7427 | 483 | 7664 | 400 | 8608 | 147 |
| | 1E-09 | 7695 | 494 | 7867 | 411 | 7734 | 587 | 7665 | 495 | 7175 | 497 | 8335 | 168 |
| | 1E-08 | 7447 | 322 | 7504 | 188 | 7484 | 401 | 7129 | 566 | 6962 | 442 | 8248 | 143 |

Supplemental Table 2. Results for mixtures of TCDD and PCB105. Mean and standard error (SE) of naïve-corrected fluorescence in the H1G1 assay (n=3).

| | | PCB105 [M] | | | | | | | | | | | |
|------|-------|------------|-----|-------|-----|-------|-----|-------|-----|-------|-----|-------|-----|
| | | Vh | | 1E-07 | | 5E-07 | | 1E-06 | | 5E-06 | | 1E-05 | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Vh | | 145 | 78 | 205 | 109 | 904 | 310 | 1468 | 351 | 2475 | 327 | 1777 | 205 |
| | 1E-13 | 222 | 94 | 337 | 156 | 1036 | 266 | 1520 | 422 | 2489 | 288 | 2041 | 173 |
| | 5E-13 | 472 | 216 | 548 | 173 | 1120 | 234 | 1738 | 400 | 2631 | 350 | 2118 | 202 |
| | 1E-12 | 614 | 170 | 670 | 173 | 1338 | 263 | 1708 | 424 | 2488 | 286 | 2105 | 225 |
| TCDD | 5E-12 | 2004 | 324 | 1918 | 284 | 2122 | 340 | 2190 | 434 | 2732 | 347 | 2008 | 146 |
| [M] | 1E-11 | 3056 | 439 | 2761 | 402 | 2564 | 323 | 2548 | 471 | 2756 | 345 | 2068 | 327 |
| | 5E-11 | 4554 | 513 | 4388 | 616 | 3997 | 482 | 3831 | 585 | 3151 | 400 | 2124 | 315 |
| | 1E-10 | 4593 | 483 | 4677 | 814 | 4243 | 484 | 4234 | 476 | 3342 | 620 | 2098 | 411 |
| | 2E-10 | 4068 | 522 | 4470 | 345 | 4159 | 363 | 3923 | 536 | 2975 | 462 | 1899 | 226 |

Supplemental Table 3. Results for mixtures of TCDD and galangin. Mean and standard error (SE) of naïve-corrected fluorescence in the H1G1 assay (n=3).

| | | Galangin [M] | | | | | | | | | | | |
|------|-------|--------------|------|---------|------|-------|------|-------|------|-------|------|-------|-----|
| | | Vh | | 2.5E-06 | | 5E-06 | | 1E-05 | | 2E-05 | | 3E-05 | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Vh | | 260 | 24 | 1180 | 136 | 1833 | 315 | 1979 | 286 | 2462 | 292 | 2274 | 337 |
| | 1E-13 | 375 | 55 | 1319 | 195 | 2048 | 338 | 2156 | 306 | 2625 | 266 | 2441 | 394 |
| | 5E-13 | 727 | 87 | 1580 | 232 | 2283 | 293 | 2234 | 322 | 2725 | 295 | 2367 | 340 |
| | 1E-12 | 988 | 119 | 1657 | 213 | 2481 | 338 | 2442 | 415 | 2742 | 351 | 2559 | 449 |
| TCDD | 5E-12 | 3163 | 504 | 3221 | 603 | 3749 | 795 | 3190 | 792 | 2885 | 475 | 2407 | 445 |
| [M] | 1E-11 | 4669 | 832 | 4416 | 848 | 4404 | 978 | 3734 | 978 | 3117 | 453 | 2489 | 337 |
| | 5E-11 | 7019 | 1379 | 7060 | 1366 | 6745 | 1872 | 5517 | 1624 | 3950 | 890 | 2664 | 441 |
| | 1E-10 | 8366 | 1631 | 8257 | 1670 | 7848 | 1849 | 6189 | 1895 | 4189 | 1010 | 2677 | 534 |
| | 2E-10 | 7554 | 1464 | 8102 | 1512 | 7814 | 2043 | 6164 | 1627 | 3898 | 993 | 2496 | 438 |

Supplemental Table 4. Results for mixtures of TCDD and galangin (narrow range of doses).

Mean and standard error (SE) of naïve-corrected fluorescence in the H1G1 assay (n=4).

| | | Galangin [M] | | | | | | | |
|------|-------|--------------|-----|---------|-----|-------|-----|---------|-----|
| | | Vh | | 1.0E-06 | | 5E-06 | | 1.0E-05 | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Vh | | 166 | 38 | 738 | 45 | 1792 | 290 | 2178 | 392 |
| | 5E-13 | 519 | 29 | 1033 | 97 | 1917 | 340 | 2330 | 377 |
| | 1E-12 | 848 | 66 | 1287 | 99 | 2058 | 303 | 2324 | 426 |
| | 2E-12 | 1593 | 134 | 1824 | 171 | 2260 | 334 | 2438 | 364 |
| TCDD | 4E-12 | 2608 | 350 | 2664 | 284 | 2610 | 437 | 2758 | 486 |
| [M] | 6E-12 | 3347 | 318 | 3402 | 354 | 2946 | 522 | 2965 | 571 |
| | 8E-12 | 3916 | 443 | 3777 | 360 | 3341 | 583 | 3468 | 674 |
| | 1E-11 | 4238 | 483 | 3967 | 468 | 3410 | 754 | 3228 | 680 |
| | 2E-11 | 4894 | 482 | 4701 | 531 | 3796 | 819 | 3659 | 723 |

Supplemental Table 5. Results for mixtures of TCDD and DIM. Mean and standard error (SE) of naïve-corrected fluorescence in the H1G1 assay (n=3).

| | | DIM [M] | | | | | | | | | | | |
|------|---------|---------|------|-------|------|-------|------|---------|------|-------|------|-------|-----|
| | | Vh | | 1E-06 | | 5E-06 | | 7.5E-06 | | 1E-05 | | 2E-05 | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Vh | | 88 | 53 | 320 | 85 | 406 | 57 | 396 | 86 | 524 | 67 | 944 | 160 |
| | 1E-13 | 319 | 44 | 584 | 86 | 571 | 40 | 590 | 172 | 652 | 71 | 1153 | 193 |
| | 5E-13 | 531 | 104 | 722 | 145 | 655 | 83 | 693 | 123 | 681 | 76 | 1181 | 168 |
| | 1E-12 | 1041 | 71 | 1130 | 92 | 844 | 92 | 839 | 75 | 845 | 118 | 1282 | 126 |
| TCDD | 5E-12 | 3991 | 490 | 3195 | 656 | 2186 | 272 | 1664 | 307 | 1467 | 221 | 1584 | 129 |
| [M] | 1E-11 | 5630 | 974 | 4716 | 770 | 3214 | 612 | 2699 | 580 | 2390 | 425 | 2001 | 106 |
| | 2.5E-11 | 7465 | 1585 | 6545 | 1366 | 4948 | 916 | 4591 | 1010 | 4127 | 899 | 2950 | 156 |
| | 5E-11 | 9195 | 1420 | 7863 | 1480 | 6452 | 1192 | 5668 | 1329 | 5257 | 1077 | 3754 | 395 |
| | 1E-10 | 9078 | 1896 | 8086 | 1316 | 6727 | 1288 | 6387 | 1556 | 5914 | 1121 | 4220 | 521 |